Use of Creatine Analogues and Creatine Kinase Modulators for the Prevention and Treatment of Obesity and Its Related Disorders

Field of invention

The present invention provides for new use for creatine compounds (creatine analogues and compounds which modulate one or more of the structural or functional components of the creatine kinase/ creatine phosphate system) as therapeutic agents. More particularly, the present invention provides a method of treating or preventing certain metabolic disorders of human and animal metabolism relating to aberrant body weight regulation as manifested in obesity and it's related disorders.

Related Applications

The present application is a continuation-in-part of and claims priority to Provisional Application U.S.S.N. 60/005,882, filed October 26, 1995, the entire disclosure of which is incorporated herein by reference.

Background of the invention

There are several metabolic diseases of human and animal metabolism, eg., obesity and severe weight loss that relate to energy imbalance-- where caloric intake versus energy expenditure -- is imbalanced. Obesity, which can be defined as a body weight more than 20% in excess of the ideal body weight, is a major health problem in Western affluent societies. It is associated with an increased risk for cardiovascular disease, hypertension, diabetes, hyperlipidaemia and an increased mortality rate. Obesity is the result of a positive energy balance, as a consequence of an increased ratio of caloric intake to energy expenditure. The molecular factors regulating food intake and body weight balance are incompletely understood. Five single-gene mutations resulting in obesity have been described in mice, implicating genetic factors in the etiology of obesity. (Friedman, j. m., and Leibel, r. l. Cell 69: 217-220 (1990)). In the ob mouse a single gene mutation, obese, results in profound obesity, which is accompanied by diabetes (Friedman,J.M.,et. al. Genomics 11: 1054-1062 (1991)). Cross- circulation experiments have suggested that the ob mice are deficient of a blood-borne factor regulating nutrient intake and energy metabolism (Coleman,D.L. Diabetologia 14: 141-148 (1978)). Using positional cloning technologies, the mouse ob gene, and subsequently its human homologue, have been recently cloned (Zhang,Y.,et. al., Nature 372: 425-432 (1994)). Daily intraperitoneal injections of either

mouse or human recombinant OB protein reduced the body weight of obese mice ob/ob by 30% after 2 weeks of injection. The protein reduced food intake and increased energy expenditure in the ob/ob mice (Halaas et. al., Science 269: 543-546 (1995)).

Cachexia on the other hand is characterized by severe weight loss and imbalanced energy expenditure, examples being patients with cancer or HIV infections.

The creatine kinase/creatine phosphate system is an energy generating system operative predominantly in the brain, muscle, heart, retina, adipose tissue and the kidney (Walliman et. al. , Biochem. J. 281: 21-40 (1992)). The components of the system include the enzyme creatine kinase (CK), the substrates creatine (Cr), creatine phosphate (CrP), ATP,ADP, and the creatine trasporter. The enzyme catalyses reversibly the transfer of a phosphoryl group from CrP to ADP to generate ATP which is the main source of energy in the cell. This system represents the most efficient way to generate energy upon rapid demand. The creatine kinase isoenzymes are found to be localized at sites where rapid rate of ATP replenishment is needed such as around ion channels and ATPase pumps. Some of the functions associated with this system include efficient regeneration of energy in the form of ATP in cells with fluctuating and high energy demand, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

The substrate Cr is a compound which is naturally occurring and is found in mammalian brain, skeletal muscle, retina, adipose tissue and the heart. It's phosphorylated form CrP is also found in the same organs and is the product of the CK reaction. Both compounds can be easily synthesized and are believed to be non toxic to man. A series of creatine analogues have also been synthesized and used as probes to study the active site of the enzyme. Kaddurah- Daouk et al. (WO 92/08456 published May 29, 1992 and WO 90/09192, published August 23, 1990; U.S. 5,321,030; and U.S. 5,324,731) described methods for inhibiting growth, transformation, or metastasis of mammalian cells using related compounds. Examples of such compounds include cyclocreatine, homocyclocreatine and beta guanidino propionic acid. These same inventors have also demonstrated the efficacy of such compounds for combating viral infections (U.S. 5,321,030). Eigebaly in U.S. Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in muscle tissue. Cohn in PCT publication No. W094/16687 describes a method for inhibiting the growth of several tumors using creatine and related compounds.

It is an object of the present invention to provide methods for treatment of metabolic diseases that relate to deregulated body weight by administering to an afflicted individual an

amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/creatine phosphate system sufficient to prevent, reduce or ameliorate the symptoms of the disease. These compounds are collectively referred to as "creatine compounds."

Summary of the Invention

The present invention provides a method of treating or preventing a metabolic disorder which relates to an imbalance in the regulation of body weight. Examples would be obesity and its related disorders (such as cardiovascular disease, hypertension, diabetes, hyperlipidaemia, osteoporosis and osteoarthritis) and severe weight loss. It consists of administering to a patient susceptible to or experiencing said disorder a creatine compound (creatine analogues and compounds which modulate one or more of the structural or functional components of the creatine kinase/creatine phosphate system) as therapeutic in the form of a pharmacologically acceptable salt as the pharmaceutical agent effective to treat or prevent the disease or condition.

Obesity is the result of a positive energy balance, as a consequence of an increased ratio of caloric intake to energy expenditure while severe weight loss is a result of a negative energy balance. The creatine kinase system is known to be involved in energy metabolism and it's substrates creatine phosphate, and ATP are among the highest energy compounds in the cell. It is now possible to modify this system and come up with compounds that can change energy balance and subsequently treat, prevent or ameliorate the diseases mentioned. One can increase energy state or decrease it by using substrates or inhibitors for the enzyme creatine kinase, or modulators of the enzyme system (compounds which modify any of its components) such as the creatine transporter.

The present invention also provides compositions containing creatine compounds in combination with a pharmaceutically acceptable carrier. Also, they could be used in combination with effective amounts of standard chemotherapeutic agents which act on regulating body weight and others to prophylactically and/or therapeutically treat a subject with a disease related to body weight control.

Packaged drugs for treating subjects having energy imbalance resulting in weight loss or gain are also the subject of the present invention. The packaged drugs include a container holding the creatine compound, in combination with a pharmaceutically acceptable carrier, along with

instructions for administering the same for the purpose of preventing, ameliorating, arresting or eliminating a disease related to glucose level regulation.

By treatment is meant the amelioration or total avoidance of the metabolic disorder as described herein. By prevention is meant the avoidance of a currently recognized disease state, as described herein, in a patient evidencing some or all of the metabolic disorders described above.

For all of these purposes, any convenient route of systemic administration is employed, e.g., orally, parenterally, intranasally or intrarectally. The above compositions may be administered in a sustained release formulation. By sustained release is meant a formulation in which the drug becomes biologically available to the patient at a measured rate over a prolonged period. Such compositions are well known in the art.

Detailed Description of the Invention

The method of the present invention generally comprises administering to an individual afflicted with a disease or susceptible to a disease involving body weight regulation, an amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate symptoms of the disease. Components of the system which can be modulated include the enzyme creatine kinase, the substrates creatine, creatine phosphate, ADP, ATP, and the transporter of creatine. As used herein, the term "modulate" means to change, affect or interfere with the functioning of the components in the creatine kinase/creatine phosphate enzyme system.

The creatine kinase/creatine phosphate system is an energy generating system operative predominantly in the brain, muscle, heart, retina, adipose tissue and the kidney (Walliman et. al. , Biochem. J. 281: 21-40 (1992)). The components of the system include the enzyme creatine kinase (CK), the substrates creatine (Cr), creatine phosphate (CrP), ATP,ADP, and the creatine trasporter. The enzyme catalyses reversibly the transfer of a phosphoryl group from CrP to ADP to generate ATP which is the main source of energy in the cell. This system represents the most efficient way to generate energy upon rapid demand. The creatine kinase isoenzymes are found to be localized at sites where rapid rate of ATP replenishment is needed such as around ion channels and ATPase pumps. Some of the functions associated with this system include efficient regeneration of energy in the form of ATP in cells with fluctuating and high energy demand, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

Brown and white adipose tissue both contain creatine kinase and the substrates creatine and creatine phosphate, with activity of the enzyme 50 times higher in brown tissue (Bertlet et al., Biochim Biophys. Acta 437:166-174 (1976)). Brown fat tissue is responsible for energy expenditure and heat generation through the process of non-shivering thermogenesis. It was suggested that creatine may be involved in co-promoting mitochondrial respiration for thermogenesis.

The substrate Cr is a compound which is naturally occurring and is found in mammalian brain, skeletal muscle, retina and the heart. It's phosphorylated form CrP is also found in the same organs and is the product of the CK reaction. Both compounds can be easily synthesized and are believed to be non toxic to man. A series of creatine analogues have also been synthesized and used as probes to study the active site of the enzyme. Kaddurah- Daouk et al. (WO 92/08456 published May 29, 1992 and WO 90/09192, published August 23, 1990; U.S. 5,321,030; and U.S. 5,324,731) described methods for inhibiting growth, transformation, or metastasis of mammalian cells using related compounds. Examples of such compounds include cyclocreatine, homocyclocreatine and beta guanidino propionic acid. These same inventors have also demonstrated the efficacy of such compounds for combating viral infections (U.S. 5,321,030). Elgebaly in U.S. Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in muscle tissue. Cohn in PCT publication No. W094/16687 describes a method for inhibiting the growth of several tumors using creatine and related compounds.

The term "creatine compound" will be used herein to include creatine, and compounds which are structurally similar to it and analogues of creatine and creatine phosphate. The term "creatine compound" also includes compounds which "mimic" the activity of creatine, creatine phosphate, or creatine analogues i.e., compounds which modulate the creatine kinase system. The term "mimics" is intended to include compounds which may not be structurally similar to creatine but mimic the therapeutic activity of the creatine analogues or structurally similar compounds. The term creatine compounds will also include inhibitors of creatine kinase, ie. compounds which inhibit the activity of the enzyme creatine kinase, molecules that inhibit the creatine transporter or molecules that inhibit the binding of the enzyme to other structural proteins or enzymes or lipids. The term "modulators" of the creatine kinase system" are compounds which modulate the activity of the enzyme, or the activity of the transporter of creatine, or the ability of the enzyme to associate with other cellular components. These could be substrates for the enzyme and they would have the ability to build in their phosphorylated state intracellularly. These types of

molecules are also included in our term creatine compounds. The term creatine "analogue" is intended to include compounds which are structurally similar to creatine, compounds which are art- recognized as being analogues of creatine, and/or compounds which share the same function as creatine.

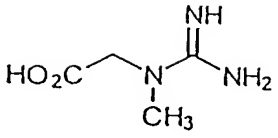
Creatine (α also known as N-(aminoiminomethyl)-N-methyl glycine; methylglycosamine or N-methyl-guanidino acetic acid is a well-known substance. (see the Merck Index, Eleventh Edition No. 2570 , 1989). Creatine is phosphorylated chemically or enzymatically to creatine kinase to generate creatine phosphate, which is also well known (see The Merck Index, No.7315). Both creatine and creatine phosphate (phosphocreatine) can be extracted from animals or tissue or synthesized chemically. Both are commercially available.

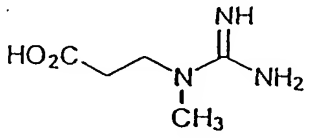
Cyclocreatine is an essentially planer cyclic analogue of creatine. Although cyclocreatine is structurally similar to creatine, the two compounds are distinguishable both kinetically and thermodynamically. Cyclocreatine is phosphorylated efficiently by the enzyme creatine kinase in the forward reaction, both in vitro and in vivo. Rowley, G.L., J.AM. Chem.Soc. 93:5542-5551 (1971); McLaughlin, A.C. et. al. J. Biol. Chem. 247, 4382-4388 (1972). It represents a class of substrate analogues of creatine kinase and which are believed to be active.

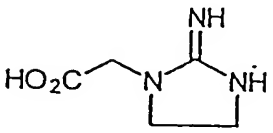
Examples of substances (creatine analogues) known or believed to modify the creatine kinase/ creatine phosphate system are listed in Tables 1 and 2.

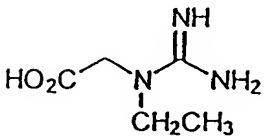
TABLE 1

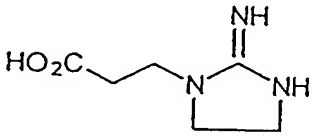
CREATINE ANALOGS

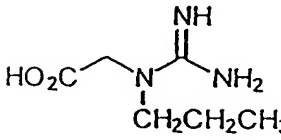


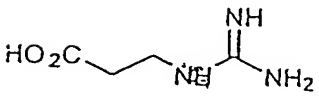


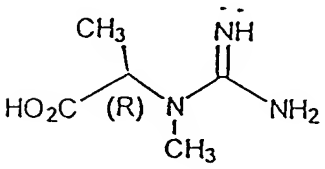


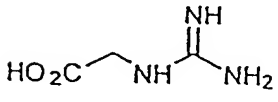


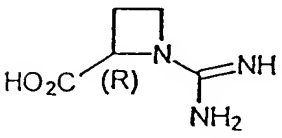


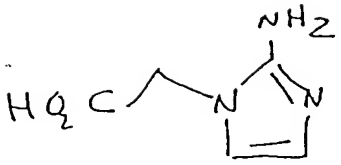


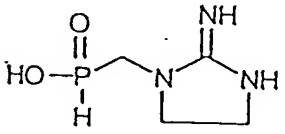


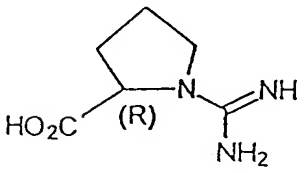


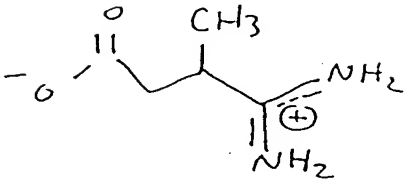


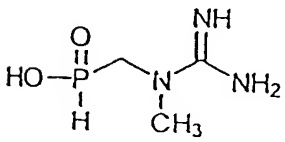


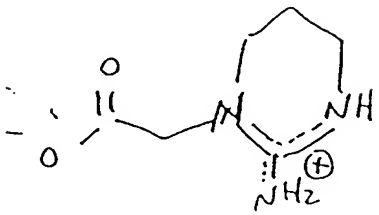












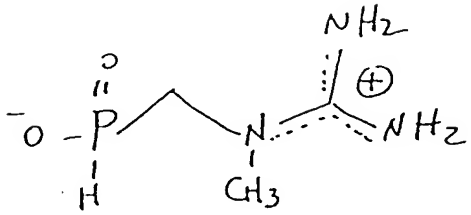
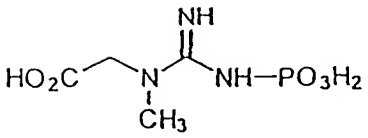
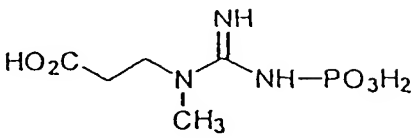
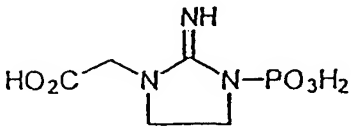


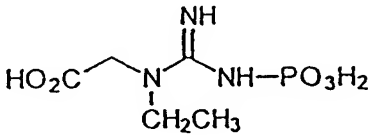
TABLE 2

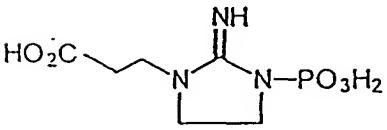
CREATINE PHOSPHATE ANALOGS

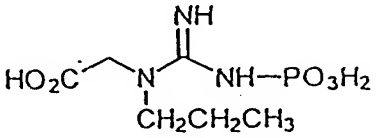


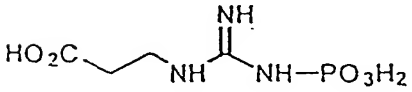


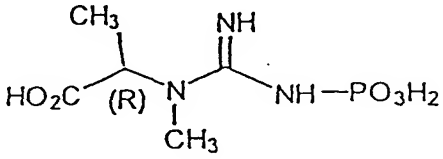


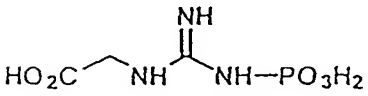


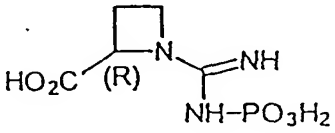


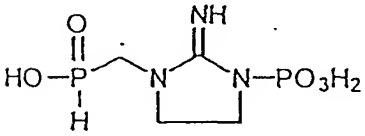


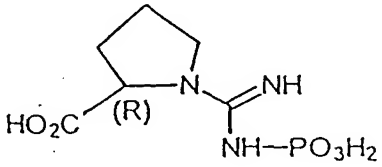


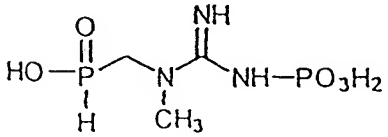


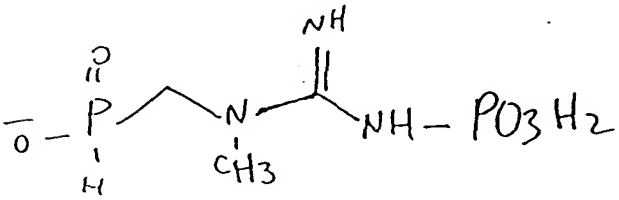


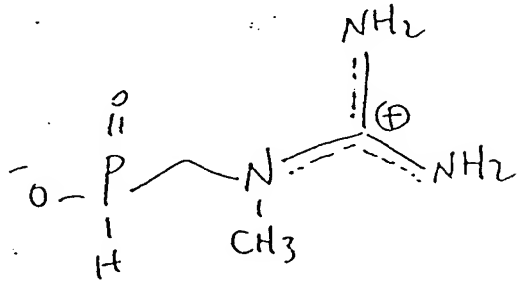










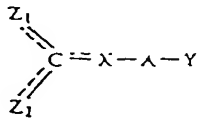


Most of these compounds have been previously synthesized for other purposes (Rowley et. al., J.Am.Chem.Soc., 93: 5542-5551, (1971); Mclaughlin et. al., J.Biol.Chem., 247: 4382-4388 (1972) Nguyen, A.C.K., " Synthesis and enzyme studies using creatine analogues", Thesis, Dept of Pharmaceutical Chemistry, Univ. Calif., San Francisco, 1983; Lowe et al., J. Biol. Chem., 225:3944-3951(1980); Roberts et. al., J. Biol. Chem., 260:13502-13508 (1995) Roberts et. al., Arch. biochem. Biophy., 220:563-571, 1983, and Griffiths et. al., J.Biol. Chem., 251: 2049-2054 (1976). The contents of all of the forementioned references are expressly incorporated by reference. Further to the forementioned references, Kaddurah-Daouk et. al., (WO 92/08456; WO 90/09192; U.S. 5,324,731; U.S. 5,321,030) also provide citations for the synthesis of a plurality of creatine analogues. The contents of all the aforementioned references and patents are incorporated herein by reference.

It will be possible to modify the substances described below to produce analogues which have enhanced characteristics, such as greater specificity for the enzyme, enhanced solubility or stability, enhanced cellular uptake, or better biding activity. Salts of products may be exchanged to other salts using standard protocols.

Bisubstrate analogues of creatine kinase and non hydrolyizable substrate analogues of creatine phosphate (non transferable moieties which mimic the N phosphoryl group of creatine phosphate) can be designed readily and would be examples of creatine kinase modulators. Creatine phosphate compounds can be synthesized chemically or enzymatically. The chemical synthesis is well known. Annesley., T.M., Walker,J.B., Biochem.Biophys.Res. Commun., 74: 185-190 (1977); Cramer, F., Scheiffele, E.;VOLLMAR, A.,Chem.Ber., 95:1670-1682 (1962).

Creatine compounds which are particularly useful in this invention include those encompassed by the following general formula:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$-NHOH, $-NO_2$, $-SO_3H$, $-C(=O)NHSO_2J$ and $-P(=O)(OH)(OJ)$, wherein J is selected from the group

consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: rromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH_2L and -$COCH_2$L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, CHR_1, CR_1, O and S, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having O-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents

independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_5-C_9 a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) 2 C_5-C_9 a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a C_5-C_9 a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =O, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =O and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) 2 C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: $-CO_2H$-NHOH, $-SO_3H$, $-NO_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6

alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1-C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -$(PO_3)_n$NMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -$(PO_3)_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of $-NHR_2$, $-CH_2R_2$ and $-NR_2OH$, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

Currently preferred compounds include cyclocreatine, creatine phosphate and those included in Tables 1 and 2 hereinabove.

The modes of administration for these compounds includes but is not limited to, oral, transdermal, or parenteral (eg., subcutaneous, intramuscular, intravenous, bolus or continuous infusion). The actual amount of drug needed will depend on factors such as the size , age and severity of disease in afflicted individual. Creatine has been given to athletes in the range of 2-8 gms /day to improve muscle function. Creatine phosphate was administered to patients with congestive heart failure also in the range of several gm/day and was very well tolerated. In experimental animal models of cancer or viral infections, were creatine compounds were shown to be active, amounts of 1gm/kg/day were needed intraveniously or intraperitoneially. For this invention the creatine compound will be administered at dosages and for periods of time effective to reduce, ameliorate or eliminate the symptoms of the disease. Dose regimens may be adjusted for purposes of improving the therapeutic or prophylactic response of the compound. For example, several divided doses may be administered daily, one dose, or cyclic administration of the compounds to achieve the required therapeutic result.

The creatine compounds can be formulated according to the selected route of administration. The addition of gelatin, flavoring agents, or coating material can be used for oral applications. For solutions or emulsions in general, carriers may include aqueous or alcoholic/ aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride, potassium chloride among others. In addition intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers among others.

Preservatives and other additives can also be present. For example, antimicrobial, antioxidant, chelating agents, and inert gases can be added (see, generally, Remington's Pharmaceutical Sciences, 16th Edition, Mack, 1980).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentati many equivalents to the specific embodiments of the invention described herein. Such equivalents a intended to be encompassed by the following claims.

What is claimed is: